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SEGREGATION STUDIES OF ISOZYME VARIATION IN *METAPHIDIPPUS GALATHEA* (ARANEAE, SALTICIDAE)[1]

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ABSTRACT. Three field-collected isofemale lines of *Metaphidippus galathea* were established as laboratory colonies. The female parents and their progeny were electrophoretically examined for 13 proteins coding for 21 isozymes. Eight proteins were segregating for allozymes and four were analyzed for Mendelian inheritance. Although sample sizes were small, the GOT-1 locus showed a tendency toward deviation from the expected inheritance pattern. Nonconformance to genetic expectations may be due to multiple-mating, to selection effects from laboratory rearing conditions, or to genetic drift.

Several studies now claim to show varying levels of genetic variation in spiders based on allozyme surveys (Steiner et al. in prep.; Roeloffs & Riechert 1988; Terranova & Roach 1987a; Smith 1986; Lubin & Crozier 1985; Cesaroni et al. 1981; Manchenko 1981). Genetic variation is important for the study of phenomena such as population differentiation and interpopulation migration (Roeloffs & Riechert 1988; Smith 1986; Lubin & Crozier 1985) and in defining taxonomic differences (Terranova & Roach 1987b; Pennington 1979). For the studies to be valid representatives of genetic variability, it is important that genetic inheritance of the allozymes be verified, as it is possible for enzyme variation to be environmentally induced (Gerasimova & Smirnova 1986; Arnason & Chambers 1987) or to be found only during ontogeny (Korochkin & Matveeva 1974). In this paper, we report the first evidence based on parental-offspring correlations to support Mendelian inheritance of allozymes in spiders.

METHODS

Three female *Metaphidippus galathea* were collected in mid-June of 1984 at the University of Missouri's Tucker Natural Prairie in central Missouri. The Prairie is a tallgrass remnant located 25 km east of Columbia, Missouri (Drew 1947). The females were returned to the Biological Control of Insects Research Laboratory, United States Department of Agriculture, Agricultural Research Service, where they were housed in self-watering cages (Jackson 1974), fed early-mid 5th instar *Trichoplusia ni* (Hubner) larvae for maintenance, and kept at approximate relative humidities and temperatures of 70% and 25 °C, respectively. Approximately 17 days later all three were observed with egg cases. Sixteen days after that the egg cases hatched and the spiderlings were maintained on *Drosophila melanogaster* Meigen. At 60 days of age, the spiderlings and their maternal parents were frozen for electrophoresis. Aging spiders for 60 days reduces the possibility of ontogenic effects on electrophoretic pattern.

Starch gel electrophoresis was performed and the resulting gels stained for enzyme activity as described in Steiner and Joslyn (1979). Individuals were run side-by-side on 1 cm thick gels which could be horizontally-sliced into 1 mm slices for protein histochemical staining. The protein systems analyzed are listed in Table 1. Two electrode chamber-gel buffer systems were used. The first was a pH 8.2 LiOH/Boric Acid electrode buffer with a pH 8.5 Trizma Base/citric acid gel used to analyze the proteins ACPH, ALDOX, EST, PGM, and PGI. The second contained citric acid and Trizma Base in both the electrode chamber (pH 8.2) and the gel (pH 8.4) and was used to analyze ADK, GOT, G-3-PDH, HK, IDH, α-GPDH, MDH and 6-PGDH. Material from the female and her offspring were run on the same gels to aid in recognizing isozyme banding homologies. The resulting segregation

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Table 1.—Enzymatic loci and their electrophoretic characteristics for the spider, *Metaphidippus galathea.* Abbreviations: DH = dehydrogenase; E.C.N. = enzyme classification number assigned by the International Union of Pure and Applied Chemistry; * = polymorphic protein system; S = protein quaternary structure, M = monomer and D = dimer; *N* = number of loci observed for the individual protein (the total is 21); relative distance is the anodal migration distance measured from the origin to the band on a 12% Sigma starch gel run at 70 mA/gel for 12 h on the designated system in METHODS. For GOT-2, the protein migrated toward the cathode 3 mm from the origin.

Protein	Enzyme Classification Number	Abbrev.	S	*N*	Relative distance in mm
Acid phosphatase	3.1.3.2	ACPH	M	2	18 and 15
Adenylate kinase	2.7.4.3	ADK	M	1	24
Aldehyde oxidase	1.2.3.1	ALDOX	M	1	22
Esterase	3.1.1.1	EST*	M	4	68, 60, 50 and 33
Glutamate oxaloacetate transaminase	2.6.1.1	GOT*	D	2	16 and −3
Glyceraldehyde-3-phosphate DH	1.2.1.12	G-3-PDH	—	1	16
alpha-Glycerophosphate DH	1.1.1.8	α-GPDH*	D	2	20 and 10
Hexokinase	2.7.1.1	HK	—	1	2
Isocitrate DH	1.1.1.42	IDH*	D	2	12 and 8
Malate DH	1.1.1.37	MDH	—	2	20 and 8
6-phosphogluconate DH	1.1.1.44	6-PGDH*	D	1	17
Phosphoglucomutase	2.7.5.1	PGM	—	1	31
Phosphoglucose isomerase	5.3.1.9	PGI	—	1	5

data were analyzed using the χ^2 test with Yates Correction Factor for small sample sizes.

RESULTS

A total of 21 loci encoding 13 proteins were identified. Eight loci were polymorphic, but four of these were esterases which we have not included in this analysis. This was because some individuals expressed overlap of alleles between the esterase loci making genotype assignments difficult. These esterase genes expressed the highest variation found.

The other four loci included GOT-1 (migrating to 16 mm), IDH-1 (migrating to 12 mm), α-GPDH-1 (migrating to 20 mm) and 6-PGDH (migrating to 17 mm). Heterozygotes of all these loci were triple-banded while homozygotes were single-banded suggesting a dimeric enzyme structure consisting of two polypeptide chains (Table 1).

In this study the maternal genotypes could be determined directly from the gels, leading us to infer the paternal genotype. This enabled us to generate an expected genotype ratio in the F_1, assuming a single-pair mating took place. The assumptions of male genotype and F_1 genotype ratio were met at all loci although GOT-1 approached a significant departure. At that locus, a deficiency of heterozygotes and an excess of the common allele homozygote occurred which was strengthened when similar mating types were pooled. Assuming an alternative ratio of 1:2:1 only led to a higher χ^2 value ($\chi^2 = 15.73$, $P < 0.001$) due to a lack of GOT-1^{44} and decreased expected numbers of GOT-1^{55}.

DISCUSSION

The segregation patterns we observed fit Mendelian expectations. This is expected, since Mendelian inheritance of allozyme genes is widely reported in the literature for *Drosophila*, humans, plants and other organisms.

Only the segregation at GOT-1 approaches a significant departure ($\chi^2 = 2.77$, $P = 0.105$) which can be explained in several ways. First, the GOT-1 results may be anomalous since we eannot completely exclude sampling error due to small samples (genetic drift). Only further study can verify or falsify this argument, although the strength of the χ^2 statistic which includes the correction for small sample sizes, and the relatively higher number of surviving F_1 would not seem to support it. Or, it may be that selection is favoring GOT-1^{55} under the laboratory conditions. However, it remains difficult to bring the observed ratio into a 1:1 balance by just invoking selection against or for a single genotype, and the question arises as to what the selective faetor

Table 2.—Segregation statistics for four allozyme loci in the spider, *Metaphidippus galathea*. Parental genotypic crosses are indicated by A, B, and C. Sex ratio refers to female to male with female genotype known and male genotype inferred. Expected F_1 genotypes are calculated from the expected genotypic ratio. In the genotype codings, 5 designates the most commonly occurring band, 1–4 indicates faster migrating bands, and 6–9 indicates slower migrating bands. We assume bands are indicative of the allelic state. For the chi-square tests, matings with similar parental genotypes were pooled when individual chi-square tests were insignificant. Lack of significant differences between observed and expected numbers of progeny is designated NS.

Locus		Parental genotypes	F_1 sex ratio	F_1 genotypes, obs/exp			Expected F_1 genotypic ratio	χ^2
				45	55	56		
GOT-1	A	55 × 45	5:4	2/4	7/5		1:1	NS
	B	55 × 45	1:4	1/2	3/2		1:1	NS
	C	45 × 55	3:4	2/2	2/2		1:1	NS
6-PGDH	A	56 × 55	5:4		4/4	5/4	1:1	NS
	B	55 × 55	1:4		4/4		Fixed	
	C	55 × 55	3:4		7/7		Fixed	
α-GPDH-1	A	55 × 56	5:4		5/5	4/5	1:1	NS
	B	55 × 56	3:4		2/3	5/4	1:1	NS
	C	55 × 55	1:4		4/4		Fixed	
IDH-1	A	55 × 55	5:4		9/9		Fixed	
	B	56 × 55	1:4		1/2	3/2	1:1	NS
	C	55 × 55	3:4		7/7		Fixed	

might be, assuming laboratory conditions are more optimal to survival than natural conditions. Obviously the other allozyme loci do not reflect a selection pattern. It is also possible that sperm competition is involved. In fact, Jackson (1980) has found evidence for sperm competition in *Phidippus*, suggesting evidence for both multiple mating and pre-zygotic selection. In our case, assuming that GOT-1 somehow plays a role in any pre-zygotic selection further complicates the issue.

An alternative explanation is that GOT-1 female A (Table 2) may have mated with two males rather than one. Fertilization by an additional GOT-1^{55} genotypic male would make half the female's progeny homozygous; removing that half from the nine offspring would then result in a 1:1 ratio of offspring resulting from the mating with the GOT-1^{45} heterozygous male.

Although the evidence for either multiple-mating and/or sperm competition is admittedly weak, the suggestion that electrophoresis can be used as a tool to study reproductive strategies in spiders is appropriate. This approach should be considered especially in view of Jackson's (1980) research. Questions concerning the extent of multiple mating and the survival or fitness qualities of the paternal parent could then be assessed. In this way, population biology parameters previously undefined could lead to a better understanding of spider ecology, behavior and evolution.

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